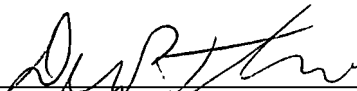


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PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Group No.: 1614

Didier Cataldo, et al.

Serial No.: 10/594,101

Filed: May 22, 2007

For: USE OF A TRIOXOPYRIMIDINE FOR THE TREATMENT AND PREVENTION OF BRONCHIAL INFLAMMATORY DISEASES

TRANSMITTAL OF CERTIFIED COPY

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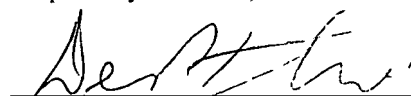
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<u>Country</u>	<u>Application No.</u>	<u>Filing Date</u>
Europe	04007920.4	April 1, 2004

Respectfully submitted,



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**Patentanmeldung Nr.    Patent application No.    Demande de brevet n°**

04007920.4

Der Präsident des Europäischen Patentamts;  
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets  
p.o.

**R C van Dijk**



Anmeldung Nr:  
Application no.: 04007920.4  
Demande no:

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Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:  
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.  
If no title is shown please refer to the description.  
Si aucun titre n'est indiqué se référer à la description.)

Use of a trioxypyrimidine for the treatment and prevention of bronchial  
inflammatory diseases

In Anspruch genommene Priorität(en) / Priority(ies) claimed /Priorité(s)  
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## Use of a trioxypyrimidine for the treatment and prevention of bronchial inflammatory diseases

The present invention relates to the use of a trioxypyrimidine compound for the treatment and prevention of bronchial inflammatory diseases.

### Introduction

- 5 Matrix metalloproteases (MMPs) are a family of zinc- and calcium-dependent proteases that are capable of degrading the extracellular matrix (ECM) and basement membrane (Egeblad, M., and Werb, Z., Nat. Rev. Cancer 2 (2002) 161-174; Overall, C.M., and Lopez-Otin, C., Nat. Rev. Cancer 2 (2002) 657-672). They are believed to have pivotal roles in embryonic development and growth
- 10 (Holmbeck, K., et al., Cell 99 (1999) 81-92; Vu, T.H., et al., Cell 93 (1998) 411-422) as well as in tissue remodeling and repair (Shapiro, S.D., Curr. Opin. Cell Biol. 10 (1998) 602-608; Lund, L.R., et al., EMBO J. 18 (1999) 4645-4656). Excessive or inappropriate expression of MMPs may therefore contribute to the pathogenesis of many tissue-destructive processes, including tumor progression (Egeblad, M., and Werb, Z., Nat. Rev. Cancer 2 (2002) 161-174; Overall, C.M., and Lopez-Otin, C., Nat. Rev. Cancer 2 (2002) 657-672) and aneurysm formation (Carmeliet, P., et al., Nat. Genet. 17 (1997) 439-444). MMP effects are far from being restricted to ECM degradation (Chang, C., and Werb, D., Trends Cell Biol. 11 (2001) S37-43). Peptide growth factors that are sequestered by ECM proteins become available once
- 20 degraded by MMP-9 (Manes, S., et al., J. Biol. Chem. 274 (1999) 6935-6945). MMPs can increase the bioavailability of VEGF (Bergers, G., et al., Nat. Cell Biol. 2 (2000) 737-744) but also generate angiogenesis inhibitors such as angiostatin by cleavage of plasminogen (Dong, Z., et al., Cell 88 (1997) 801-810).
- 25 Inhibition of MMPs, either with the naturally occurring Tissue Inhibitors of Metalloproteases (TIMPs), or with low molecular weight inhibitors, resulted in impressive anti-tumor and anti-metastatic effects in animal models (Brown, P.D., Med. Oncol. 14 (1997) 1-10). Most of the low-molecular weight inhibitors of MMPs are derived from the hydroxamic acid compound class and inhibit MMPs in a broad manner, being not selective for MMP-2 and MMP-9, the key MMPs in
- 30 tumor invasion, metastatic spread, and angiogenesis. However, MMP inhibiting molecules from another structural class, the trioxypyrimidines, have been

described, e.g. in WO 97/23465 and WO 01/25217. This class of compounds is extremely potent, and highly selective, with an almost exclusive specificity for MMP-2, MMP-9, while sparing most other members of the MMP family of proteases.

5 Several MMP inhibitors, predominantly of the hydroxamic acid substance class with broad substrate specificity were, and in part still are, in clinical testing for anti-tumor treatment. All of the published clinical results with these inhibitors were disappointing, showing little or no clinical efficacy (Fletcher, L., Nat. Biotechnol. 18 (2000) 1138-1139). The reason for this lack of efficacy in the clinic most likely is the  
10 fact that patients could not be given high enough doses for anti-tumor or anti-metastatic activity because of the side effects associated with these broadly acting inhibitors. These dose-limiting side effects were predominantly arthralgias and myalgias (Drummond, A.H., et al., Ann. N.Y. Acad. Sci. 878 (1999) 228-235). As a possible way to circumvent this problem, the combination of MMP inhibitors with  
15 classical cytostatic/cytotoxic compounds was evaluated in animal studies. Indeed, in these experiments, MMP inhibitors, in combination with cytostatic/cytotoxic drugs, showed enhanced tumor inhibiting efficacy (Giavazzi, R., et al., Clin. Cancer Res. 4 (1998) 985-992). In addition, International Patent Application No. PCT/EP02/04744 shows the combination of trioxypyrimidine based gelatinase  
20 inhibitors and cytotoxic/cytostatic compounds such as cisplatin, Paclitaxel, Gemcitabine or Etoposide.

There have been made a lot of attempts to identify compounds which prevent or inhibit bronchial inflammatory diseases. Inhaled synthetic glucocorticosteroids are widely used in the treatment of bronchial asthma where they provide very effective  
25 first line treatment. However, a range of unwanted side effects and the often complex dosing schedules associated with these drugs frequently result in poor patient compliance. Lofeprednol etabonate, an inactive metabolite soft steroid, is being examined in clinical trials for its effects on airway inflammation (Szelenyi, I., et al., Drugs Today 36 (2000) 313-320). Ciclesonide, a pro-drug soft steroid, has  
30 demonstrated efficacy without side effects in a once daily formulation in asthma patients and is being developed for the treatment of both asthma and chronic obstructive pulmonary disease (Rohatagi, S., et al., J. Clin. Pharmacol. 43 (2003) 365-378).

Therefore, there exists a need for highly potent substances which can be used for the treatment or prevention of bronchial inflammatory diseases.

### Description of the Invention

5 It was surprisingly found that trioxypyrimidine-based MMP inhibitors which are highly selective for MMP-2, MMP-9 and MMP-14 are useful for the treatment or prevention of bronchial inflammatory diseases.

10 The invention therefore provides the use of a trioxypyrimidine compound having an inhibitory activity against MMP-1, MMP-2, MMP-3, MMP-9 and MMP-14 defined as

- a) an  $IC_{50}$  value of less than 5  $\mu M$  for MMP-2, MMP-9 and MMP-14 each;
- b) a ratio of more than 100 for the  $IC_{50}$  values of MMP-1:MMP-2, MMP-1:MMP-9, MMP-1:MMP-14; and
- 15 c) a ratio of more than 10 for the  $IC_{50}$  values of MMP-3:MMP-2, MMP-3:MMP-9, MMP-3:MMP-14,

for the treatment or prevention of bronchial inflammatory diseases in a host mammal in need of such treatment.

20  $IC_{50}$  values are measured by an in vitro assay for MMP enzymatic activity. Such an assay is described by Stack, M.S., and Gray, R.D., J. Biol. Chem. 264 (1989) 4277-4281. This assay is based on the determination of MMP enzymatic activity on a dinitrophenol substrate and fluorescence measurement of the substrate after cleaving by MMPs.

25 The invention further provides the use of such trioxypyrimidine compounds for the manufacturing of a medicament for the treatment of bronchial inflammatory diseases in a patient suffering from such a disease.

Matrix metalloproteinases are well-known in the state of the art and are defined, e.g., by their EC numbers (MMP-1 EC 3.4.24.7; MMP-2 EC 3.4.24.24; MMP-3 EC 3.4.24.17, MMP-9 EC 3.4.24.35, MMP-14 EC 3.4.24).

5 Trioxypyrimidines useful for the invention are compounds from a well-known structural class. Such compounds are described in, for example, US Patent Nos. 6,242,455 and 6,110,924; WO 97/23465, WO 98/58915, WO 01/25217, which are incorporated herein by reference, and Grams, F., et al., Biol. Chem. 382 (2001) 1277-1285, and are effective and highly selective for MMP-2, MMP-9, and MMP-14.

According to the invention, the following compounds are particularly preferred:

5-Biphenyl-4-yl-5-[4-(4-nitro-phenyl)-piperazin-1-yl]pyrimidine-2,4,6-trione  
(Compound I)

10 5-(4-Phenoxy-phenyl)-5-(4-pyrimidin-2-yl-piperazin-1-yl)-pyrimidine-2,4,6-trione  
(Compound II)

15 5-[4-(4-Chloro-phenoxy)-phenyl]-5-(4-pyrimidin-2-yl-piperazin-1-yl)-pyrimidine-2,4,6-trione  
(Compound III)

5-[4-(3,4-Dichloro-phenoxy)-phenyl]-5-(4-pyrimidin-2-yl-piperazin-1-yl)-pyrimidine-2,4,6-trione  
(Compound IV)

20 5-[4-(4-Bromo-phenoxy)-phenyl]-5-(4-pyrimidin-2-yl-piperazin-1-yl)-pyrimidine-2,4,6-trione  
(Compound V).

25 According to the invention the trioxypyrimidine-based inhibitors have to be administered to the patient suffering from such a disease, over several months or years (especially in case of prevention), to the patient in need of such a therapy. The trioxypyrimidine compounds are administered preferably as sprays, with non-toxic doses ranging between micro and nanomolar concentrations.

The invention relates to a method used for treating bronchial inflammatory diseases, preferably asthma and chronic obstructive pulmonary disease (COPD) in

a host mammal in need of such treatment, e.g., a patient suffering from such a disease, by the application of a trioxypyrimidine compound according to the invention to a patient in a pharmaceutically effective amount. Asthma is an inflammatory disease of the bronchial tree related or not to an allergen exposure.

5 This inflammation provokes symptoms in patients by stimulating the bronchial smooth muscles to contract, enhancing the mucus secretion, and inducing bronchial morphological changes thought to be an aggravating factor regarding the course of the disease. Airway hyperresponsiveness is a hallmark of the disease and is responsible for most of symptoms. Bronchial tree is a very complex tissue with  
10 many cell types (epithelial cells, smooth muscle cells, inflammatory cells, nerves, mucus producing cells, fibroblasts, and the like) and the bronchial remodelling events which comprise many aspects mainly consist in a deposition of extracellular matrix components in the bronchial walls and an hyperplasia of the mucus producing cells. The use of trioxypyrimidine compounds according to the  
15 invention inhibits the inflammatory cells influx in the compartments of bronchoalveolar lavage and peribronchial tissue and inhibits the hyperresponsiveness defined as an abnormal response to stimulating agents such as methacholine. The disease and current treatments are reviewed in, e.g., GINA Workshop Report, Global Strategy for Asthma Management and Prevention (NIH Publication No. 02-3659) and Fabbri, L.M., and Hurd, S.S., Eur. Respir. J. 22 (2003) 1-2.

The invention therefore further relates to a method for treating bronchial inflammatory diseases in a patient suffering from such a disease, using a trioxypyrimidine compound according to the invention in a therapeutically  
25 effective amount.

The invention preferably further relates to a method for treating emphysema in a patient suffering from such a disease, using trioxypyrimidine compounds according to the invention. In such a disease, the alveolar walls are destroyed by proteolytic processes and this destruction impairs the transfer of oxygen to the blood.  
30 Physiological problems also occurs because of the derived hyperinflation which causes abnormalities in the ventilation by causing a dysfunction of respiratory muscles and because of a hypertension in pulmonary arteries leading to cardiac failure in advanced stages.



According to the invention the trioxypyrimidine compound has to be administered over several months or years, to the patient in need of such a therapy. The trioxypyrimidine compounds are administered preferably by the aerosolization of a liquid or powder formulation, with non-toxic doses ranging between micro and nanomolar concentrations per kg and day.

The exact dosage of the MMP inhibitors will vary, but can be easily determined. In general, the preferred daily dosage of the inhibitors will range between 1  $\mu\text{mol/kg}$  and day to 100 nmol/kg and day.

The pharmaceutical compositions are aqueous compositions having physiological compatibility. The compositions include, in addition, auxiliary substances, buffers, preservatives, solvents and/or viscosity modulating agents. Appropriate buffer systems are based on sodium phosphate, sodium acetate or sodium borate. Preservatives are required to prevent microbial contamination of the pharmaceutical composition during use. Suitable preservatives are, for example, benzalkonium chloride, chlorobutanol, methylparabene, propylparabene, phenylethyl alcohol, sorbic acid. Such preservatives are used typically in an amount of 0.01 to 1% weight/volume.

Suitable auxiliary substances and pharmaceutical formulations are described in Remington's Pharmaceutical Sciences, 16th ed., 1980, Mack Publishing Co., edited by Oslo et al. Typically, an appropriate amount of a pharmaceutically-acceptable salt is used in the formulation to render the formulation isotonic. Examples of a pharmaceutically acceptable substances include saline, Ringer's solution and dextrose solution. The pH of the solution is preferably from about 5 to about 8, and more preferably from about 7 to about 7.5.

A further preferred object of the invention is a pharmaceutical composition of a trioxypyrimidine according to the invention for the treatment of bronchial inflammatory diseases, and its use, containing a trioxypyrimidine-cyclodextrin complex (inclusion of the trioxypyrimidine in the cyclodextrin) formed of such a trioxypyrimidine or a salt thereof and a cyclodextrin, preferably a water-soluble cyclodextrin derivative (water soluble being defined as a solubility of at least 0.5 g/100ml water at 25°C). In the complex, preferably 1 mol of trioxypyrimidine is

complexed and enclosed by 1 mol of  $\alpha$ ,  $\beta$ - or  $\gamma$ -cyclodextrin or a derivative thereof. The preferred cyclodextrins are

- alpha-cyclodextrin and its synthetic derivatives such as HP $\alpha$ CD, methylated  $\alpha$ CD, hydroxybutyl  $\alpha$ CD, maltosyl  $\alpha$ CD, glucosyl  $\alpha$ CD.
- 5    - beta-cyclodextrin and its synthetic derivatives such as HP $\beta$ CD, SBE $\beta$ CD, RM $\beta$ CD, DIME $\beta$ CD, TRIME $\beta$ CD, hydroxybutyl  $\beta$ CD, glucosyl  $\beta$ CD, maltosyl  $\beta$ CD.
- gamma-cyclodextrin and its synthetic derivatives such as HP $\gamma$ CD, RM $\gamma$ CD and DIME $\gamma$ CD, hydroxybutyl  $\gamma$ CD, glucosyl  $\gamma$ CD, maltosyl  $\gamma$ CD.

10

Cyclodextrins useful according to the invention are cyclic oligosaccharides produced by enzymatic degradation of starch, which are composed of a variable number of glucopyranose units, mostly 6, 7 or 8: these cyclodextrins are respectively named  $\alpha$ ,  $\beta$ , and  $\gamma$  cyclodextrins ( $\alpha$ CD,  $\beta$ CD and  $\gamma$ CD). Cyclodextrins  
15 according to the invention are cyclodextrins per se or cyclodextrin derivatives, which are at least water soluble in an amount of 0.5 gr/100ml at 25°C.

15

The water-soluble cyclodextrin derivative preferably used in the present invention refers to a derivative having water solubility of at least that of  $\beta$ -cyclodextrin. Examples of such water-soluble cyclodextrin derivatives are sulfobutylcyclodextrin,  
20 hydroxypropylcyclodextrin, maltosylcyclodextrin, and salts thereof. In particular, sulfobutyl- $\beta$ -cyclodextrin, hydroxypropyl- $\beta$ -cyclodextrin, maltosyl- $\beta$ -cyclodextrin, and salts thereof

20

Derivatives preferred according to the invention are also methylcyclodextrins (products of the cyclodextrins methylation), dimethylcyclodextrins (DIMEB)  
25 (preferably substituted in 2 and in 6), trimethylcyclodextrins (preferably substituted in 2, 3 and 6), "random methylated" cyclodextrins (RAMEB) (preferably substituted at random in 2, 3 and 6, but with a number of 1,7 to 1,9 methyl by unit glucopyranose), hydroxypropylcyclodextrins (HPCD, hydroxypropylated cyclodextrins preferably substituted randomly mainly in position 2 and 3 (HP- $\beta$ CD, HP- $\gamma$  CD)), sulfobutylethercyclodextrins (SBECD), hydroxyethyl-  
30 cyclodextrins, carboxymethylethylcyclodextrins, ethylcyclodextrins, cyclodextrins amphiphiles obtained by grafting hydrocarbonated chains in the hydroxyl groups and being able to form nanoparticles, cholesterol cyclodextrins and triglycerides-

25

30

cyclodextrins obtained by grafting cyclodextrins monoaminated (with a spacer arm).

5 The trioxypyrimidine-cyclodextrin complex of the present invention may be obtained by producing an aqueous solution containing a trioxypyrimidine compound thereof and a water-soluble cyclodextrin derivative. The water-soluble cyclodextrin derivative is used in an amount of preferably one mol or more per one mol trioxypyrimidine, more preferably 1-10 mol, and particularly preferably 1-2 mol cyclodextrin per mol trioxypyrimidine.

10 The higher the concentration of the water-soluble cyclodextrin derivative, the more the solubility of the trioxypyrimidine increases. No particular limitation is imposed on the method for producing the aqueous solution, and for example it is produced by use of water or a buffer in a temperature range approximately from -5 to 35° C.

15 When a cyclodextrins aqueous solution is stirred with an excess of a trioxypyrimidine, there is a complex formation between these two molecules. The filtration of this solution allows recovering the complex a trioxypyrimidine in solution in the filtrate, the complex being soluble in water. The complex can also be obtained by mixing a solubilized known quantity of a trioxypyrimidine in aqueous solution with a solubilized known quantity of CD by calculating the adequate proportions.

20 Another way of obtaining a complex is to add a solution of a trioxypyrimidine in a solvent (e. g. alcohol, acetone, etc) to a cyclodextrin aqueous solution. The complex can be formed after sufficient stirring, either after evaporation of the solvent, or even in the presence of the solvent.

25 The lyophilization or the nebulization of solutions of the complex according to the invention allows the complex to be obtained in solid form. One can thus obtain a complex in the form of an amorphous powder. It is also possible to obtain the complex in the solid state after dissolution of CD and a trioxypyrimidine in an appropriate organic solvent and further evaporation of the solvent.

30 Other methods can be used for solid complexes preparation which are violent stirring of a suspension of a trioxypyrimidine and CD in a very small quantity of

water, then complex collecting after drying or the use of CO<sub>2</sub> in a supercritical state for mixing a trioxypyrimidine and CD in presence of CO<sub>2</sub> in a supercritical state.

5 The complex according to the present invention can be prepared, for example, in a manner known per se from a solution or using the paste method, where the weight ratio of cyclodextrin to trioxypyrimidine must be between 2 to 540, and is preferably between 2 to 25, particularly preferably in the region of 2.6 to 3.5 (for a 1:1 complex with cyclodextrin) or of 5.2 to 6.2 (for a 1:2 complex with cyclodextrin).

10 It is preferred to prepare the complex from a concentrated, aqueous cyclodextrin preparation. The cyclodextrin concentration of the preparation is preferably between 50 and 400 mM. Preference is given to a cyclodextrin concentration of from 100 to 250 mM. Depending on the consistency, the mixtures are intensively stirred or kneaded. The percent by weight of the cyclodextrin is based upon the total weight of the aqueous cyclodextrin preparation.

15 The reaction temperature is usually between 20° C and 80° C, preferably between 20° C and 60° C, particularly preferably between 25° C and 45° C. The reaction time depends on the temperature and is between one hour and some days. Preference is given to a reaction time of at least 7 days to reach equilibrium of complex formation.

20 According to the invention it has been established that complexes between a trioxypyrimidine and a cyclodextrin increase the solubility of a trioxypyrimidine in water amazingly. It is also apparent that the formation of the complex did not interfere with the pharmacological properties of the trioxypyrimidine.

25 All these properties allow to prepare liquid formulations as solutions for injection or for nebulization and allow the bioavailability to be improved, in particular orally. A trioxypyrimidine-cyclodextrin complex of the present invention may be used as such or in a powder form which is obtained by removing co-existing water. Examples of the method for removing water include lyophilization and drying under reduced pressure. A powder product obtained from lyophilization is  
30 particularly preferred.

5 The trioxypyrimidine-cyclodextrin complex of the present invention exhibits its effects through either oral administration or parenteral administration, and it is preferably formed into a formulation for parenteral administration, particularly an injection formulation or topical administration, particularly an aerosol formulation.

Examples of the form of formulation include tablets, capsules, powders, and granules. These may be produced through a known technique by use of typical additives such as excipients, lubricants, and binders.

10 A preferred pharmaceutical formulation for nebulization contains trioxypyrimidine, cyclodextrin (CD), NaCl and water. Especially preferred is a combination of (for 200 ml of solution):  
Trioxypyrimidine 0.05-0.2 g, preferably 0.1 g; 10-50 g CD, preferably 20 g CD, preferably HP $\beta$ CD; sodium chloride 1.2-1.5 g, preferably 1.42 g (isotonicity) and  
15 water, preferably pyrogen-free, sterile, purified water ad 200 ml.

Such a formulation is useful for the treatment of bronchial inflammatory diseases.

The solution is prepared by dissolving CD in 100 ml of purified water, adding trioxypyrimidine and NaCl by stirring so as to dissolve them and complete with water so as to obtain 200 ml of solution. Preferably the solution is sterilized by  
20 filtration through a 0.22  $\mu$ m polypropylene membrane or by a steam sterilization process.

The following examples, references, and figures are provided to aid the understanding of the present invention, the true scope of which is set forth in the appended claims. It is understood that modifications can be made in the  
25 procedures set forth without departing from the spirit of the invention.

### Description of the Figures

- 5      **Figure 1**      Effects of intraperitoneal injection of a Compound Isuspension on BAL eosinophil counts (Fig 1a) and peribronchial inflammation score (fig 1b). Controls are mice exposed only to PBS and not allergen (PBS) and mice exposed to ova by inhalation and placebo by intraperitoneal injection (OVA)
- 10      **Figure 2**      Therapeutic effects of Compound I-HP- $\beta$ -CD complex, fluticasone and placebo (PLAC) administered by aerosols on BAL eosinophilia (2a), peribronchial inflammation score (2b), and tissue eosinophils infiltration score (2c) in a short term (5 days) allergen exposure model.
- 15      **Figure 3**      Therapeutic effects of Compound I-HP- $\beta$ -CD complex, fluticasone and placebo (PBS) administered by aerosols on BAL eosinophilia (3a), peribronchial inflammation score (3b), and tissue eosinophils infiltration score (3c) in a long term (11 weeks) allergen exposure model. Mice sensitized but unexposed to allergens (PBS) and mice sensitized and exposed to OVA (PLAC) were treated by PBS inhalation.

### Example 1

20      **Preparation of a soluble complex of compound I and cyclodextrin (CD)**

- 1.1      Weigh 20 mg of compound I. Add 2 ml of solution of HP $\beta$ CD 200 mM. Stir for 24 h at 37°C. Filter in Millipore filter Millex HV 0.45  $\mu$ m. The solution obtained after filtration contains the complex compound I-CD in solution.
- 25      1.2.      Weigh 2.5 mg of compound I. Add 2 ml of solution of HP $\beta$ CD 200 mM. Stir at 37°C for 24 h or until compound I is completely dissolved. The solution obtained in this way contains the complex compound I-CD.

## Example 2

### **Determination of MMP enzymatic activity**

Inhibitors were tested in a modified fluorescence-assay as described by Stack, M.S., and Gray, R.D., J. Biol. Chem. 264 (1989) 4277-4281. Human MMP-1, MMP-2, MMP-3, MMP-9 and MMP-14 are commercially available (e.g. Calbiochem). The pro-enzymes were activated with 1 mM APMA (incubation for 30 min at 37°C) immediately before testing. Activated enzyme is diluted to 100 ng/ml in incubation buffer (50 mM Tris, 100 mM NaCl, 10mM CaCl<sub>2</sub>, pH 7.6). The compounds were dissolved in 100% DMSO. For IC<sub>50</sub> determination a minimum of 8 dilution steps between 0.5 - 1000 nM have been prepared. DNP-substrate (Bachem M1855, 255µM) was dissolved in incubation buffer.

The test tube contains 970µl incubation buffer, 10µl inhibitor solution and 10µl enzyme solution. The reaction was started by adding the 10µl substrate solution.

Kinetics of activity were determined using excitation at 280 nm and emission at 346 nm measured on a FluoroMax™ (Spex Industries Inc., Edison, NJ, USA) over 120 sec. DMSO has been used as control instead of inhibitor solution.

IC<sub>50</sub>'s are defined as the concentration of inhibitor that gives a signal that is 50% of the positive enzyme control.

IC<sub>50</sub> values (nM) are shown in Table 1.

Table 1

	MMP-1	MMP-2	MMP-3	MMP-9	MMP-14
Compound I [nM]	53,000	65	3,500	260	1
Batimastat [nM]	25	32	67	23	19

### **Example 3**

#### **Pharmaceutical compositions**

Different compositions of formulations are given for example non-exhaustively.

A preferred example for an injectable formulation is:

- 5      - HP- $\beta$ CD 200 mM; Compound I 1 mg/ml; Sterile water for injection q.s.

For 25 ml of solution:

a)      **Preparation of the solution:**

- 10      Weigh 6.77 g of HP $\beta$ CD (4.2 % of H<sub>2</sub>O) and dissolve them in 25 ml of water by injection. Add 25 mg of compound I and heat in a water bath until the latter is completely dissolved. Sterilize the solution by filtration.

b)      **Characteristics of the solution:**

The solution osmolality is 308 mOs/kg. The pH is 7.2.

The concentration of compound I and/or of CD can be modified in function of the requirements. It is preferred to adjust the tonicity by addition of NaCl.

- 15      A preferred formulation for nebulization is:

For 200 ml of solution:

- |    |  |                      |
|----|--|----------------------|
| 20 | - Compound I                             | 0.1 g                |
|    | - HP $\beta$ CD exempt from pyrogenic    | 20.15 g (75mM)       |
|    | - Sodium chloride                        | 1.42 g (isotonicity) |
|    | - Pyrogen-free, sterile, purified water, | q.s. ad 200 ml       |

- 25      a) Weigh 20.15 g of HP $\beta$ CD exempt from pyrogenic (3.2 % H<sub>2</sub>O, ROQUETTE) and dissolve them in 100 ml of purified water.
- b) Weigh 0.1 g of compound I, and 1.42 g of sodium chloride and add them to solution (a) by energetically stirring so as to dissolve them.
- c) Complete with water so as to obtain 200 ml of solution.
- d) Sterilize by filtration through a 0.22  $\mu$ m polypropylene membrane.



#### Example 4

5 Use of formulations containing compound I and HP $\beta$ CD for therapy of allergen-induced airway inflammation and bronchial hyperresponsiveness in a mouse model of asthma.

#### Materials

10 HP- $\beta$ -CD (degree of substitution = 0.64) originates from Roquette (France). Apyrogenic phosphate buffered saline (PBS) was purchased from Bio-Wittaker (Verviers, Belgium) and methacholine from Sigma-Aldrich (Germany). All other materials were of analytical grade. Sterile water for injection was used throughout this study. Sterile, apyrogenic and isotonic CD solutions were prepared at 20, 50 and 75 mM. A commercially available fluticasone solution for inhalation (Flixotide<sup>®</sup> 1mg/ml) was purchased from Glaxo-Smithkline (Genval, Belgium)

#### Sensitization, allergen exposure and therapeutic protocols

15 In order to study the modulation of airway inflammation by intraperitoneal injection of Compound I, mice were sensitized with 10  $\mu$ g ovalbumin alumin-adsorbed (aluminject, perbio, Erembodegem, Belgium) injected intraperitoneally at days 0 and 7 and were subsequently exposed to OVA 1% or PBS aerosols for 30 minutes from day 21 to 24. Intraperitoneal injections were performed 30 min  
20 before OVA inhalations. The different injected formulations were: cremophor 10%-DMSO 10%-PBS 80%- Compound I 30 mg/kg (suspension); cremophor 10%-DMSO 10%-PBS 80%- Compound I 3.75 mg/kg (solution); HP $\beta$ CD 200 mM Compound I 7.5 mg/kg (solution); HP $\beta$ CD 200 mM. All results were compared to mice sensitized and exposed to PBS and OVA treated with PBS injected  
25 intraperitoneally. In order to study the modulation of airway inflammation by inhaled Compound I, mice were sensitized as described previously. Two protocols referred to as short exposure challenge and long-term exposure challenge were used. In the short exposure challenge, mice were exposed to aerosols of Compound I- complex at concentrations of 0.03 and 0.3 mg/ml of active compound in aqueous  
30 solution of from day 21 to 27 during 30 min in a Plexiglas exposure chamber (30 x 20 x 15 cm). Mice were exposed to OVA aerosols 30 minutes after the Compound I inhalation from day 23 to 27. In the so called long-term inhalation challenge, mice

were exposed to aerosols of Compound Iat concentrations of 0.03 and 0.3 mg/ml complexed with HP $\beta$ CD in an aqueous solution during 30 min five days odd weeks and to OVA aerosols 3 days odd weeks for 11 weeks. No inhalations were performed during even weeks.

- 5        The aerosol were produced by using an ultrasonic nebuliser SYSTAM (Système Assistance Medical, Le Ledat, France), the vibration frequency of which is 2.4 MHz with variable vibration intensity and ventilation levels. Vibration intensity was fixed in position 6 and the ventilation level was 25( $v_{1/2}$ ) l/min.

#### Airway responsiveness measurement

- 10       Twenty-four hours after the last allergen exposure, the bronchial hyper responsiveness was determined by measuring the Penh using a barometric plethysmograph as proposed by Hamelmann, E., et al., Am. J Respir. Crit. Care Med. 156 (1997) 766-775). The Penh was measured at baseline and 5 min after the inhalation of increasing doses (25, 50, 75 and 100 mM) of methacholine (Mch).

#### 15       Bronchoalveolar lavage (BAL) and histology

- Immediately after the assessment of airway responsiveness, mice were sacrificed and 1 ml of PBS free of ionised calcium and magnesium but supplemented with 0.05 mM sodium EDTA was instilled 4 times via a tracheal cannula and recovered by gentle manual aspiration. The recovered bronchoalveolar lavage fluid (BAL) was
- 20       centrifuged (1800 rpm for 10 min at 4°C). The cell pellet was washed twice and finally resuspended in 1 ml of PBS. A total cell count was performed in a Thoma chamber and the differential cell counts on at least 400 cells were performed on cytocentrifuged preparations (Cytospin 2; Cytospin, Shandon td., Runcorn, Cheshire, U.K.) using standard morphologic criteria after staining with Diff-Quick
- 25       (Dade, Germany). After BAL, the thorax was opened and the left main bronchus was clamped. The left lung was excised and frozen immediately in liquid N<sub>2</sub> for protein chemistry and mRNA extraction while the right lung was processed for histology. As previously described (Cataldo, D.D., et al, Am. J. Pathol. 161 (2002) 491-498), the right lung was infused with 4% paraformaldehyde and embedded in
- 30       paraffin. Sections of 5  $\mu$ m thickness from all lobes were stained with haematoxylin and eosin. The extent of peribronchial infiltrates was estimated by an inflammation

score. Slides were coded and the peribronchial inflammation was graded in a blinded fashion using a reproducible scoring system described elsewhere (Cataldo, D.D., et al, Am. J. Pathol. 161 (2002) 491-498). A value from 0 to 3 per criteria was adjudged to each tissue section scored. A value of 0 was adjudged when no inflammation was detectable, a value of 1 for occasional cuffing with inflammatory cells, a value of 2 when most bronchi were surrounded by a thin layer (1 to 5 cells) of inflammatory cells and a value of 3 when most bronchi were surrounded by a thick layer (>5 cells) of inflammatory cells. As 5-7 randomly selected tissue sections per mouse were scored, inflammation scores could be expressed as a mean value per animal and could be compared between groups. Another score referred to as tissue eosinophil infiltration score, specifically reflecting the amounts of eosinophils infiltrating the bronchial walls, was measured as follows: after a congo red staining, seven bronchi were studied per mouse. The eosinophils were counted around the bronchi within the limits of the airway wall, the perimeter of the epithelial basement membrane was measured and the results were expressed as number of eosinophils/mm of basement membrane. The left lung was snap frozen in liquid nitrogen and crushed using a Mikro-Dismembrator S (Braun Biotech International, Melsungen, Germany) and the extracts stored at -80°C before studied. Kidneys were excised and paraffin embedded, sections of 5 µm were stained by haematoxylin and eosin. Blood was sampled by cardiac puncture and serum was stored at -80°C until analysis were performed.

All in vivo manipulations were approved by the local Veterinarian Ethics Committee.

#### Intraperitoneal injection of Compound I

The intraperitoneal injection of Compound I (either solution or precipitate) lowered the allergen-induced airway eosinophilic inflammation in BAL at doses of 3.75 to 30 mg/kg when compared to placebo (Figure 1a). At the same doses, the peribronchial inflammation scores were also significantly lowered by Compound I with an equal efficacy of all tested formulations (Figure 1b). The tissue eosinophil infiltration score was significantly lowered by the intraperitoneal injection of Compound I at doses of 7.5 and 25 mg/kg.

### Inhalational exposure to Compound I and Compound I- HP $\beta$ CD complexes.

5 The intrinsic activity of Compound I was firstly assessed as a topically active anti-inflammatory agent by using a solution of Compound I 40mg/ml in pure DMSO in a short-term exposure. When compared to the inhalation of DMSO alone, the inhalation of this formulation led to a significant decrease of BAL eosinophils ( $p<0.005$ ), peribronchial inflammation scores ( $p<0.01$ ), as well as bronchial hyperresponsiveness ( $p<0.05$ ).

10 In the short-term exposure protocol, we assessed the effects of HP- $\beta$ -CD Compound I complexes containing formulations on the airway inflammation and hyperresponsiveness. The effects of inhalation of Compound I- HP $\beta$ CD complex containing formulations were compared with those of placebo (PBS) or fluticasone (1mg/ml) used as reference therapy. Inhalation of those formulations containing Compound I at doses of 0.03 and 0.3 mg/ml induced a significant decrease in eosinophilic inflammation in BAL in an extent comparable to that of fluticasone when compared to placebo ( $p<0.0001$ ) (Figure 2a). Peribronchial inflammation scores were also lowered when compared to placebo ( $p<0.0001$ ) (Figure 2b), as well as the tissue eosinophil infiltration score ( $p<0.01$ ) (Figure 2c).

20 After long term allergen exposure, BAL eosinophilia was significantly decreased after treatment by inhalation of Compound I- HP $\beta$ CD containing formulations ( $p<0.001$ ) in the same extent as that of fluticasone (Figure 3a). The peribronchial inflammation score was also significantly decreased by inhalation of Compound I- HP $\beta$ CD containing formulations as well as by fluticasone ( $p<0.0001$ ) (Figure 3b). The tissue eosinophil infiltration score was also decreased after treatment by Compound I inhalation in an extent comparable to the fluticasone treated mice ( $p<0.01$ ) (Figure 3c).

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Patent Claims

1. Use of a trioxypyrimidine compound having an inhibitory activity against MMP-1, MMP-2, MMP-3, MMP-9 and MMP-14 defined as

- 5           a) an  $IC_{50}$  value of less than 5  $\mu$ M for MMP-2, MMP-9 and MMP-14 each;  
          b) a ratio of more than 100 for the  $IC_{50}$  values of MMP-1:MMP-2, MMP-1:MMP-9, MMP-1:MMP-14; and  
          c) a ratio of more than 10 for the  $IC_{50}$  values of MMP-3:MMP-2, MMP-3:MMP-9, MMP-3:MMP-14,

10           for the manufacturing of a medicament for the treatment of bronchial inflammatory diseases in a host mammal in need of such treatment.

2. Use according to claim 1, characterized in that said trioxypyrimidine compound is selected from the group consisting of

- 15           5-Biphenyl-4-yl-5-[4-(4-nitro-phenyl)-piperazin-1-yl]pyrimidine-2,4,6-trione  
          5-(4-Phenoxy-phenyl)-5-(4-pyrimidin-2-yl-piperazin-1-yl)-pyrimidine-2,4,6-trione  
          5-[4-(4-Chloro-phenoxy)-phenyl]-5-(4-pyrimidin-2-yl-piperazin-1-yl)-pyrimidine-2,4,6-trione  
20           5-[4-(3,4-Dichloro-phenoxy)-phenyl]-5-(4-pyrimidin-2-yl-piperazin-1-yl)-pyrimidine-2,4,6-trione  
          5-[4-(4-Bromo-phenoxy)-phenyl]-5-(4-pyrimidin-2-yl-piperazin-1-yl)-pyrimidine-2,4,6-trione.

- 25           3. Use according to claim 1 or 2, characterized in that the trioxypyrimidine compound is complexed with a water-soluble cyclodextrin.

4. Use of a trioxypyrimidine compound having an inhibitory activity against MMP-1, MMP-2, MMP-3, MMP-9 and MMP-14 defined as

- a) an  $IC_{50}$  value of less than 5  $\mu$ M for MMP-2, MMP-9 and MMP-14 each;

- b) a ratio of more than 100 for the IC<sub>50</sub> values of MMP-1:MMP-2, MMP-1: MMP-9, MMP-1:MMP-14; and
- c) a ratio of more than 10 for the IC<sub>50</sub> values of MMP-3:MMP-2, MMP-3: MMP-9, MMP-3:MMP-14,

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for the treatment or prevention of bronchial inflammatory diseases in a host mammal in need of such treatment.

5. Use according to claim 3, characterized in that said trioxypyrimidine compound is selected from the group consisting of

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5-Biphenyl-4-yl-5-[4-(4-nitro-phenyl)-piperazin-1-yl]pyrimidine-2,4,6-trione

5-(4-Phenoxy-phenyl)-5-(4-pyrimidin-2-yl-piperazin-1-yl)-pyrimidine-2,4,6-trione

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5-[4-(4-Chloro-phenoxy)-phenyl]-5-(4-pyrimidin-2-yl-piperazin-1-yl)-pyrimidine-2,4,6-trione

5-[4-(3,4-Dichloro-phenoxy)-phenyl]-5-(4-pyrimidin-2-yl-piperazin-1-yl)-pyrimidine-2,4,6-trione

5-[4-(4-Bromo-phenoxy)-phenyl]-5-(4-pyrimidin-2-yl-piperazin-1-yl)-pyrimidine-2,4,6-trione.

20

6. Use according to claim 4 or 5, characterized in that the trioxypyrimidine compound is complexed with a water-soluble cyclodextrin.

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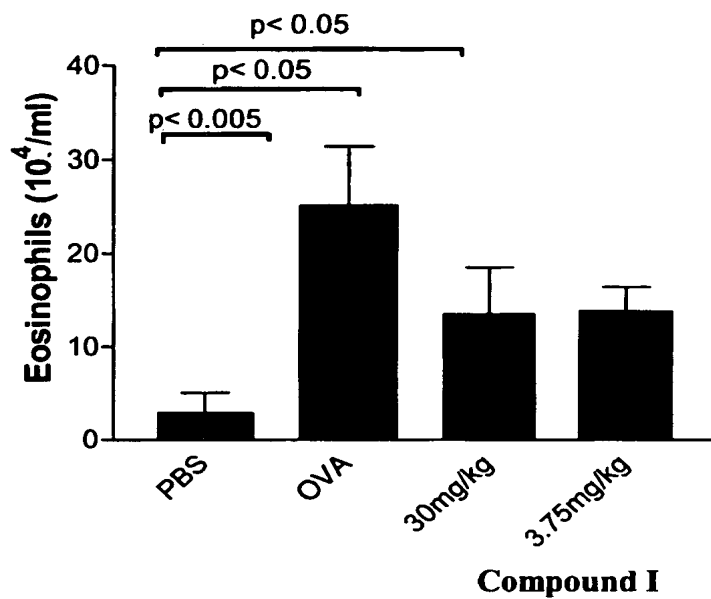
Abstract

5 The invention provides the use of a trioxypyrimidine compound having an inhibitory activity against MMP-1, MMP-2, MMP-3, MMP-9 and MMP-14 defined as a) an  $IC_{50}$  value of less than 5  $\mu M$  for MMP-2, MMP-9 and MMP-14 each; b) a ratio of more than 100 for the  $IC_{50}$  values of MMP-1:MMP-2, MMP-1: MMP-9, MMP-1:MMP-14; and c) a ratio of more than 10 for the  $IC_{50}$  values of MMP-3:MMP-2, MMP-3: MMP-9, MMP-3:MMP-14, for the manufacturing of a medicament for the treatment or prevention of bronchial inflammatory diseases.

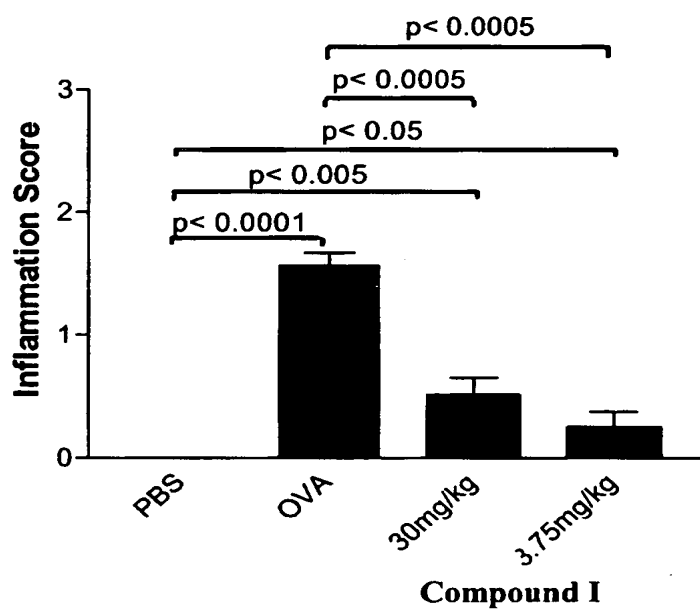


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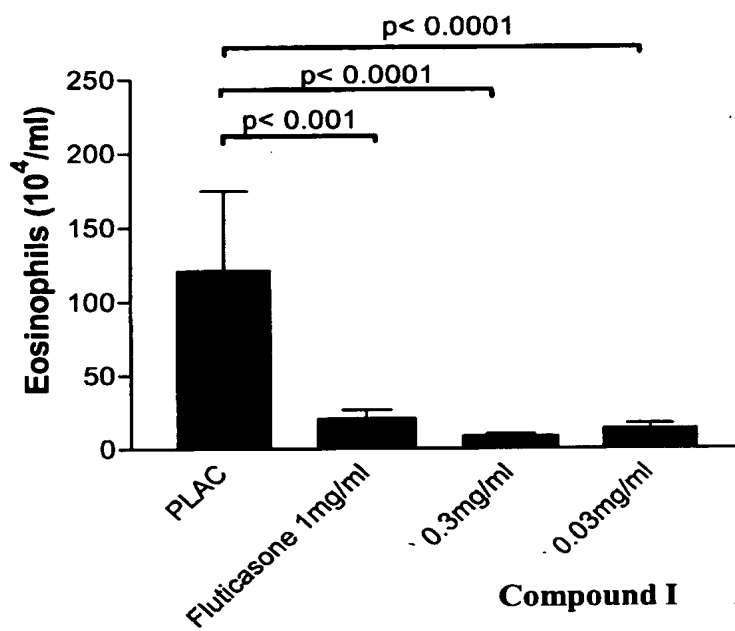
**Fig. 1a**



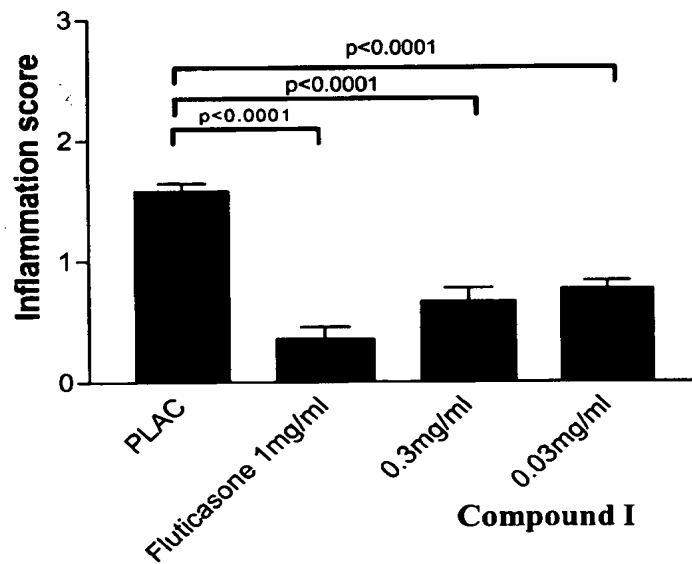
**Fig. 1b**



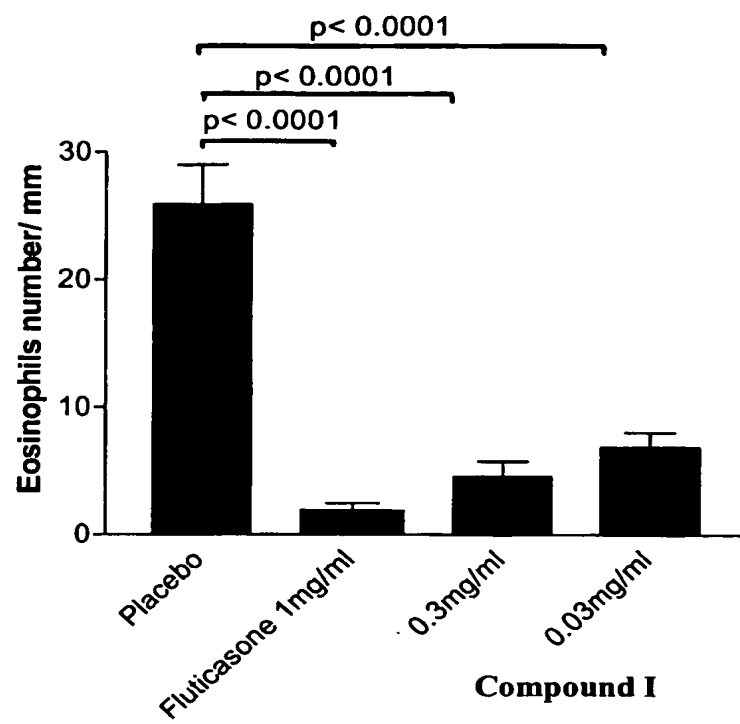
**Fig. 2a**



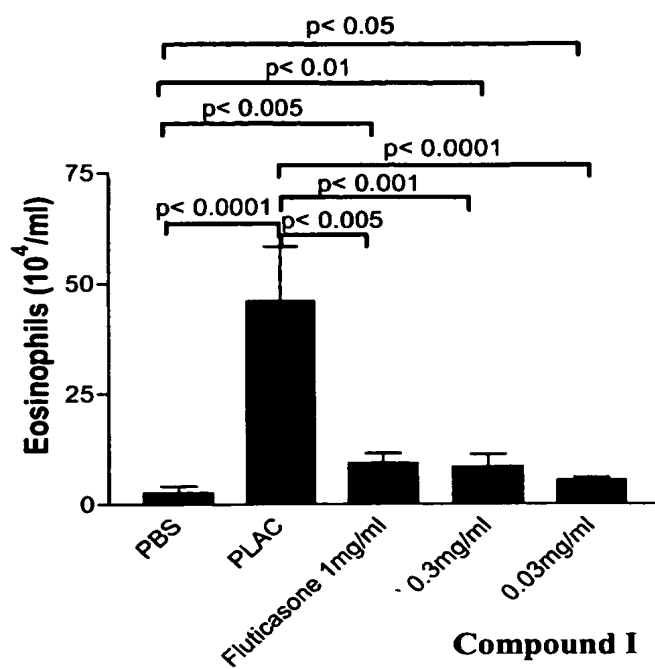
**Fig. 2b**



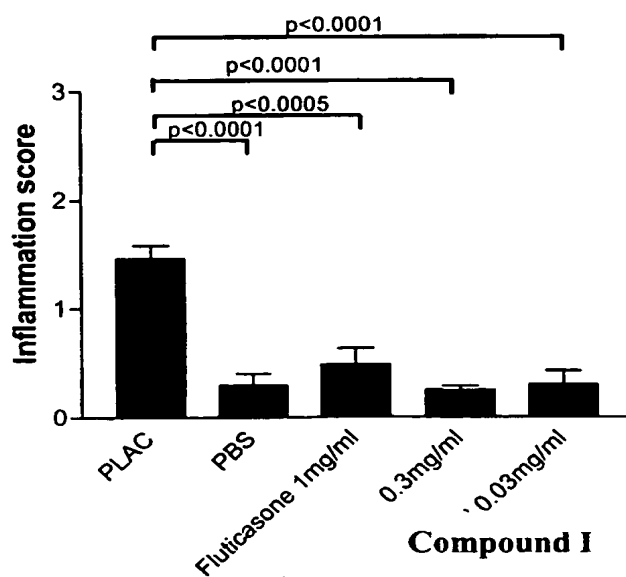
**Fig. 2c**



**Fig. 3a**



**Fig. 3b**



**Fig. 3c**

